
जल एवं अपशिष्ट जल के नमूने लेने और
परीक्षण (भौतिक एवं रसायनिक) की पद्धतियाँ

भाग 43 फिनॉल

अनुभाग 1 क्लोरोफॉर्म निष्कर्षण विधि के साथ और बिना
4-अमीनोएंटीपायरिन विधि

(दूसरा पुनरीक्षण)

Methods of Sampling and Test
(Physical and Chemical) for
Water and Wastewater

Part 43 Phenol

Section 1 4-Aminoantipyrine method with and without
Chloroform Extraction method

(Second Revision)

ICS 13.060.50

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FOREWORD

‘This Indian Standard (Part 43/Sec 1) (Second Revision) was adopted by the Bureau of Indian Standards after the draft finalized by the Water Quality Sectional Committee had been approved by the Chemical Division Council’.

Phenols, defined as hydroxy derivatives of benzene and its condensed nuclei may occur in domestic and industrial wastewaters, natural waters and portable water supplies. Chlorination of such waters may produce odoriferous and objectionable tasting chloro-phenols. Phenol removal processes in water treatment include super chlorination, chlorine dioxide or chloramine treatment, ozonation and activated carbon adsorption.

The Technical Committee responsible for formulation of IS 3025 : 1964 ‘Methods of sampling and test (physical and chemical) for water used in industry’ decided to revise the standard and publish it in separate parts. This standard superseded **57** of IS 3025: 1964 and was one among the different parts published under IS 3025 series of standards. The first revision of this standard was published in 1992 which prescribed two methods for detection of phenol in water and wastewater.

In this revision, amendment 1 and amendment 2 have been incorporated. Moreover, the following modifications have been made:

- a) This standard has been renumbered as Part 43/Sec 1 as other sections will be developed for instrument based methods;
- b) Wavelength for absorption measurement has been updated;
- c) Preparation of bromate – bromide solution has been added; and
- d) Volume of distillate has been updated in **6.1**.

This Section of Part 43 covers 4-Aminoantipyrine method with and without chloroform extraction, spectrophotometric methods for the determination of phenol in natural waters, potable water supplies, domestic and industrial wastewaters. Section 2 of Part 43 covers the flow analysis (CFA and FIA) method for determination of phenol in natural waters, potable water supplies, domestic and industrial wastewaters.

In the preparation of this standard, considerable assistance has been derived from the method No. 5530 C and D of Standard Methods for the Examination of Water and Wastewater, published by the American Public Health Association, Washington, USA, 23rd Edition, 2017.

The composition of the committee, responsible for the formulation of this standard is given at Annex A.

In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall done in accordance with IS 2 : 1960 ‘Rules for rounding off numerical values (*revised*)’.

Indian Standard

METHODS OF SAMPLING AND TEST (PHYSICAL AND CHEMICAL) FOR WATER AND WASTEWATER

PART 43 PHENOL
**Section 1 4-Aminoantipyrine method with and without
chloroform extraction method**
*(Second Revision)***1 SCOPE**

1.1 This standard (Part 43/Sec 1) prescribes two spectrophotometric methods for the determination of phenol in natural waters, potable water supplies, domestic and industrial wastewaters:

- a) 4-Aminoantipyrine method without chloroform extraction; and
- b) 4-Aminoantipyrine method with chloroform extraction.

1.2 It does not apply to differentiate between various kinds of phenols.

2 REFERENCES

The following standards contain provisions which through reference in this text constitute provisions of this standard. At the time of publications, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

<i>IS No.</i>	<i>Title</i>
7022 (Part 1) : 1973	Glossary of terms relating to water, sewage and industrial effluents: Part 1
7022 (Part 2) : 1979	Glossary of terms relating to water, sewage and industrial effluents: Part 2

3 TERMINOLOGY

For the purpose of this standard, definitions given in IS 7022 (Part 1) and IS 7022 (Part 2) shall apply.

4 SAMPLE PRESERVATION AND STORAGE

4.1 Phenol concentration usually observed in wastewater is subject to biological and chemical oxidation. Preserve and store samples at 4 °C or lower but do not allow freezing unless analysed within 4 h after collection.

4.2 After acidification with phosphoric acid to pH 4 or slightly below, the sample can be stored up to 4 weeks at 4 °C.

**5 4-AMINOANTIPYRINE METHOD
WITHOUT CHLOROFORM EXTRACTION**
5.1 Principle

Most steam distillable phenols react with 4-aminoantipyrine at pH 7.9 ± 0.1 in the presence of potassium ferricyanide to form a coloured antipyrine dye. This absorbance is measured at 500 nm.

This method is recommended for concentrations more than 1 mg/l phenol which does not require chloroform extraction technique.

5.2 Interferences

To eliminate or minimize the interferences, use steam-distilled sample. Phenols are distilled from non-volatile impurities because the volatilization of phenols is gradual, the distillate volume shall ultimately equal that of the original sample.

5.2.1 Preliminary Step of Steam Distillation

Measure 100 ml of sample and pour it into a beaker. Lower the pH to approximately to 4.0 with 85 per cent phosphoric acid (1 : 9) using methyl orange indicator or a pH meter. If the sample was already preserved using phosphoric acid, omit the addition of phosphoric acid again. Transfer the beaker contents to the distillation

apparatus which consists of a 1 l (or less) borosilicate glass distilling apparatus with Graham condenser. Distil 90 ml of the sample and stop the distillation. When boiling ceases, add 10 ml of warm distilled water to the distilling flask and resume distillation until 100 ml sample distillate have been collected. If the distillate is turbid, filter it through a pre-washed membrane filter. A larger volume of sample may alternatively be distilled for convenience. Distill 100 ml distilled water for preparing the distilled water blank.

5.3 Apparatus

5.3.1 Spectrophotometer, for use at 500 nm equipped with light path 1 to 5 cm.

5.3.2 pH Meter

5.4 Reagents

All reagents should be prepared with distilled water free from phenols and chlorine.

5.4.1 Stock Phenol Solution

Dissolve 100 mg phenol in freshly boiled and cooled distilled water and dilute to 100 ml with distilled water. Ordinarily this direct weighing gives a standard solution. If extreme accuracy is required, it has to be standardized.

CAUTION — TOXIC, HANDLE WITH CARE

5.4.1.1 Standardize the stock phenol solution

To 100 ml distilled water taken in a 500 ml glass stoppered conical flask, add 50 ml stock phenol solution and 10 ml of 0.1 N bromate-bromide solution. Immediately add 5 ml concentrated hydrochloric acid and swirl gently. If brown colour of free bromine does not persist, add 10 ml portions of bromate-bromide solution until it does. Keep flask stoppered and let stand for 10 min and then add approximately 1 g potassium iodide (KI). Usually four 10 ml portions of bromate-bromide solution are required if the stock phenol solution contains 1 000 mg phenol/l.

Prepare a blank in the same manner, using distilled water and 10 ml of 0.1 N bromate bromide solution. Titrate the blank and stock phenol solution with 0.025 N sodium thio-sulphate using starch solution indicator.

Calculate the concentration of stock phenol solution as follows:

$$\text{Concentration of stock phenol solution in mg/l} = 7.842 [(A \times B) - C] \text{ mg/l} \quad \dots(1)$$

where

A = titre value for blank, in ml;

B = volume of bromate-bromide solution used for stock phenol solution divided by 10, in ml; and

C = titre value for stock phenol solution, in ml.

5.4.2 Intermediate Phenol Solution

Dilute 1 ml of Stock solution to 100 ml volumetric flask to make it 1 ml = 10 µg/phenol and prepare daily.

5.4.3 Standard Phenol Solution

Dilute 10.0 ml of the intermediate phenol solution (see 5.4.2) in freshly boiled and cooled distilled water to 100 ml (1 ml = 1 µg phenol). Prepare daily.

5.4.4 Ammonium Hydroxide, 0.5 N.

Dilute 35 ml of fresh concentrated ammonium hydroxide to 1 l with distilled water.

5.4.5 Phosphate Buffer Solution

Dissolve 104.5 g of potassium hydrogen phosphate (K_2HPO_4) and 72.3 g of potassium di-hydrogen phosphate (KH_2PO_4) in distilled water and dilute to 1 l. The pH of the resulting solution should be 6.8.

5.4.6 4-Aminoantipyrine Solution

Dissolve 2.0 g of 4-aminoantipyrine in distilled water and dilute to 100 ml. Prepare daily.

5.4.7 Potassium Ferricyanide Solution

Dissolve 8.0 g of potassium ferricyanide [$K_3Fe(CN)_6$] in distilled water and dilute to 100 ml. Store in a brown glass bottle. Prepare fresh weekly.

5.4.8 Sodium Sulphate, Anhydrous

5.4.9 Bromate – Bromide Solution, 0.1 M.

Dissolve 2.784 gm anhydrous potassium borate ($KBrO_3$) in water, add 10 gm potassium bromide (KBr) crystals, dissolve and dilute to 1 000 ml with distilled water.

5.5 Procedure

5.5.1 Sample Analysis

Transfer 100 ml of sample distillate or a portion containing not more than 0.5 mg of phenol diluted to 100 ml into a 250 ml beaker. Prepare a 100 ml distilled water blank by distilling 100 ml distilled water and transferring into a 250 ml beaker. Treat the sample distillate and distilled water blank as follows:

Add 2.5 ml of 0.5 N ammonium hydroxide solution and adjust to pH 7.9 ± 0.1 with phosphate buffer. Add 1.0 ml of 4-aminoantipyrine solution, mix well, add 1 ml of potassium ferricyanide solution and mix it well. Let it stand for 15 min. Read the absorbance of sample against the blank at 500 nm.

5.5.2 Calibration Curve

Prepare a series of 100 ml phenol standards containing 100, 200, 300, 400 and 500 µg phenol from the standard phenol solution (see 5.4.2) in 250 ml beakers. Follow the next steps as for sample analysis (see 5.5.1). Prepare

a standard curve by plotting the absorbance values of standards versus corresponding phenol concentrations. Determine the slope of the curve.

5.6 Calculation

Calculate the quantity of phenol present in the sample as follows:

$$\text{Phenol, mg/l} = \frac{A \times \text{cal. factor}}{V} \quad \dots(2)$$

where

- A = absorbance of sample with respect to reagent blank;
 cal. factor = reciprocal of slope of the curve; and
 V = volume of sample distillate taken for analysis, in ml.

Alternatively the single standard can also be used and the following formula can be used:

$$\text{Phenol, mg/l} = \frac{C \times D \times 1\,000}{E \times B} \quad \dots(3)$$

where

- B = volume of original samples taken from distillate, in ml;
 C = Standard Phenol solution, in mg;
 D = absorbance of sample; and
 E = absorbance of Standard phenol solution.

6 4-AMINOANTIPYRINE METHOD WITH CHLOROFORM EXTRACTION

6.1 Principle

Most phenols react with 4-aminoantipyrine at pH 7.9 ± 0.1 in the presence of potassium ferricyanide to form a coloured antipyrine dye. This dye is extracted from water with chloroform and the absorbance is measured at 460 nm. The minimum detectable quantity is 1 µg/l in 500 ml distillate.

This method is more sensitive and is adoptable for use in water sample containing less than 1 mg /l.

6.2 Interferences

6.2.1 Interferences, such as phenol-decomposing bacteria, oxidizing and reducing substances, and alkaline pH values are dealt with by acidification. If the sample has been preserved as mentioned in 4, further acidification is not required. The interferences may be eliminated as follows:

6.2.1.1 Oxidizing agents, such as chlorine

Remove immediately after sampling by adding excess ferrous sulphate.

6.2.1.2 Sulphur compounds

Remove by acidifying to pH 4.0 with phosphoric acid and aerating briefly by stirring. This eliminates interference of gases like hydrogen sulphite and sulphur dioxide.

6.2.1.3 Oils and tars

Make an alkaline extraction by adjusting pH to 12 to 12.5 with sodium hydroxide pellets. Extract oil and tar from aqueous solution with 50 ml chloroform in three steps. Discard oil and tar containing layer. Remove excess chloroform in aqueous layer by warming on a water-bath before proceeding with the distillation step as shown in 6.3 preliminary step of steam distillation.

6.3 Preliminary Step of Steam Distillation

Measure 500 ml of sample into a beaker. Lower the pH to approximately 4.0 with 85 percent phosphoric acid solution (1 : 9). If the sample was already preserved using dilute phosphoric acid, omit the addition of phosphoric acid again. Transfer it to the distillation apparatus made up of glass, consisting of a 1 l borosilicate glass distilling apparatus with Graham condenser. Distil 450 ml of the sample and stop the distillation. When boiling ceases, add 50 ml of warm distilled water to the distilling flask and resume distillation until 500 ml have been collected. If the distillate is turbid, filter through a pre-washed membrane filter. Measure 500 ml distilled water into another beaker and distill it in the same way.

6.4 Apparatus

6.4.1 Spectrophotometer, for use at 460 nm and equipped with 1 to 10 cm cells.

6.4.2 Filter Funnels, Buchner type with fritted disc.

6.4.3 Filter Paper, alternative to Buchner type funnel, use Whatman No. 40 filter paper and anhydrous sodium sulphate for filtration of chloroform phase.

6.4.4 pH Meter

6.4.5 Separating Funnel, 1 000 ml capacity with ground glass stoppers and TFE stop cock.

6.5 Reagents

All reagents should be prepared with distilled water free from phenols and chlorine.

6.5.1 Phenol Stock Solution

Dissolve 100 mg phenol in freshly boiled and cooled distilled water and dilute to 100 ml with distilled water. Ordinarily this direct weighing gives a standard solution. If extreme accuracy is required, it has to be standardized.

CAUTION — TOXIC, HANDLE WITH CARE

6.5.1.1 Standardize the stock phenol solution (see 6.5.1) as per procedure given in 5.4.1.1.

6.5.2 Intermediate Phenol Solution

Dilute 5ml of stock phenol solution (*see* 6.5.1) in freshly boiled and cooled distilled water to 500 ml (1 ml = 10 µg phenol). Prepare daily.

6.5.3 Standard Phenol Solution

Dilute 25 ml of intermediate phenol solution (*see* 6.5.2) to 250 ml with freshly boiled and cooled distilled water (1 ml = 1 µg phenol). Prepare this solution within 2 h of use.

6.5.4 Ammonium Hydroxide, 0.5 N.

Dilute 35 ml of fresh concentrated ammonium hydroxide to 1 l with distilled water.

6.5.5 Phosphate Buffer Solution

Dissolve 104.5 g of potassium hydrogen phosphate (K_2HPO_4) and 72.3 g of potassium di-hydrogen phosphate (KH_2PO_4) in distilled water and dilute to 1 l. The pH of the resulting solution should be 6.8.

6.5.6 4-Aminoantipyrine Solution

Dissolve 2.0 g of 4-aminoantipyrine in distilled water and dilute to 100 ml. Prepare daily.

6.5.7 Potassium Ferricyanide Solution

Dissolve 8.0 g of material in water and dilute to 100 ml. Filter, if necessary and store in brown glass bottle. Prepare fresh weekly.

6.5.8 Chloroform

6.5.9 Sodium Sulphate, anhydrous.

6.5.10 Bromate – Bromide (0.1 M) Solution

Dissolve 2.784 gm anhydrous $KBrO_3$ in water, add 10 gm KBr crystals, dissolve and dilute to 1000 ml with distilled water.

6.6 Procedure

6.6.1 Sample Analysis

Transfer the sample distillate (*see* 6.3.2) or a suitable portion containing not more than 50 µg phenol, diluted to 500 ml, in a 1 l beaker. Prepare a 500 ml distilled water blank. Treat the sample and the distilled water blank as follows:

Add 12.0 ml of 0.5 N ammonium hydroxide and adjust pH to 7.9 ± 0.1 with phosphate buffer (10 ml may be

sufficient). Transfer to a 1 l separating funnel, add 3.0 ml 4-aminoantipyrine solution, mix well and add 3.0 ml of potassium ferricyanide and let colour develop for 3 min. The solution should be clear and light yellow. Extract immediately with chloroform using 25 ml for 1 to 5 cm cells and 50 ml for 10 cm cell. Let chloroform settle, shake again for 10 min add let the chloroform settle again. Filter each chloroform extract through filter paper or fritted glass funnels containing a 5 g layer of anhydrous sodium sulphate into 25 ml or 50 ml volumetric flasks. Make up the volume to 25 ml or 50 ml as the case may be with chloroform. Read absorbance of sample and standards against the blank at 460 nm.

6.6.2 Calibration Curve

Prepare a series of 500 ml phenol standards containing 5, 10, 20, 30, 40 and 50 µg phenol from the standard phenol solution (*see* 6.5.3) in 1 l beakers. Follow the next steps as for sample analysis (*see* 6.6.1). Prepare a standard curve by plotting the absorbance values of standards versus corresponding phenol concentrations. Determine the slope of the curve.

6.6.3 For infrequent analysis, prepare only one standard phenol solution. Prepare a 500 ml standard phenol solution of a strength approximately equal to the phenolic content of that portion of original sample used for final analysis. Also prepare a 500 ml distilled water blank. Measure absorbance of sample and standard phenol solution against the blank at 460 nm following procedure given in 6.6.1.

6.7 Calculation

Calculate the quantity of phenol present in the sample as follows :

$$\text{Phenol, mg/l} = \frac{A \times \text{cal. factor}}{V} \quad \dots(4)$$

where

A	= absorbance of sample with respect to reagent blank;
cal. factor	= reciprocal of slope of the curve; and
V	= volume of sample distillate taken for analysis, in ml.

ANNEX A

(Foreword)

COMMITTEE COMPOSITION

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